Targeted Contrast Enhanced Ultrasound to Detect Inflammation

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Introduction

Contrast enhanced ultrasound (CEUS) has been used previously to demonstrate perfusion at the tissue level[1]. These contrast agents consist of perfluorocarbon gas surrounded by a lipid shell, and can be bound to antibodies for cell-surface proteins in order to provide sustained image enhancement where these proteins are expressed by vascular endothelial cells[1]. Several studies have utilized targeted CEUS to investigate angiogenesis in tumors[2]. The use of targeted CEUS with an affinity for P-selectin, a protein expressed on the surface of endothelial cells during inflammation, could allow for the detection of inflammatory processes at a cellular level, potentially offering an earlier diagnosis of neural inflammation[3].

Methodology

Mice were imaged seven days after receiving a laminectomy and traumatic spinal cord injury at the T9 level. Sagittal images of the spinal cord at the T9 level were collected with a Vevo 2100 unit equipped with a 40 MHz transducer. While imaging continuously, contrast microbubbles bound with anti-P-selectin antibodies were injected via the tail vein and allowed to circulate for 10 minutes, at which point a bursting mechanism was activated on the ultrasound unit, rupturing all microbubbles within the beam profile[4]. The imaging procedure was repeated using ultrasound contrast bound to an isotype control antibody, which exhibits minimal binding interactions with cell surface antigens. Images from before and after the bursting pulse were processed using analysis software. By comparing the greyscale ultrasound images to a reference set of images taken before the contrast injection, the software generated a colored overlay of contrast distribution. A region of interest (ROI) was traced around the T9 spinal cord segment, which allowed for graphs to be generated, showing the variation in contrast signal amplitude in this region during the 10 minute scanning period.

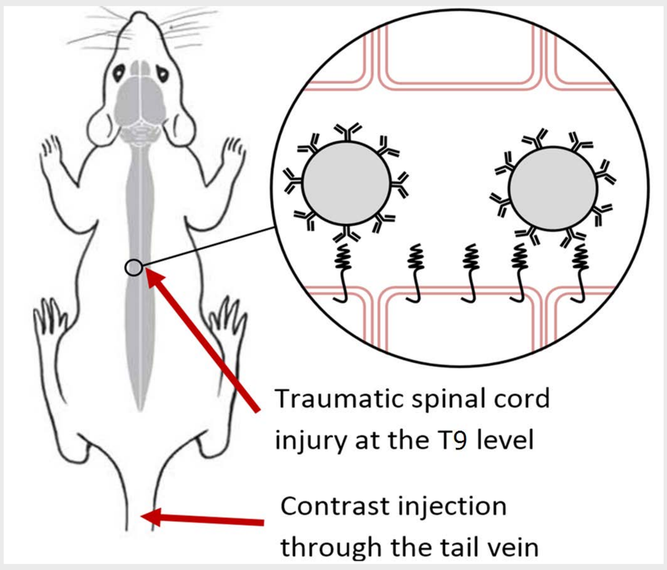


Figure 1. Diagram of experimental intervention which induces neural inflammation.

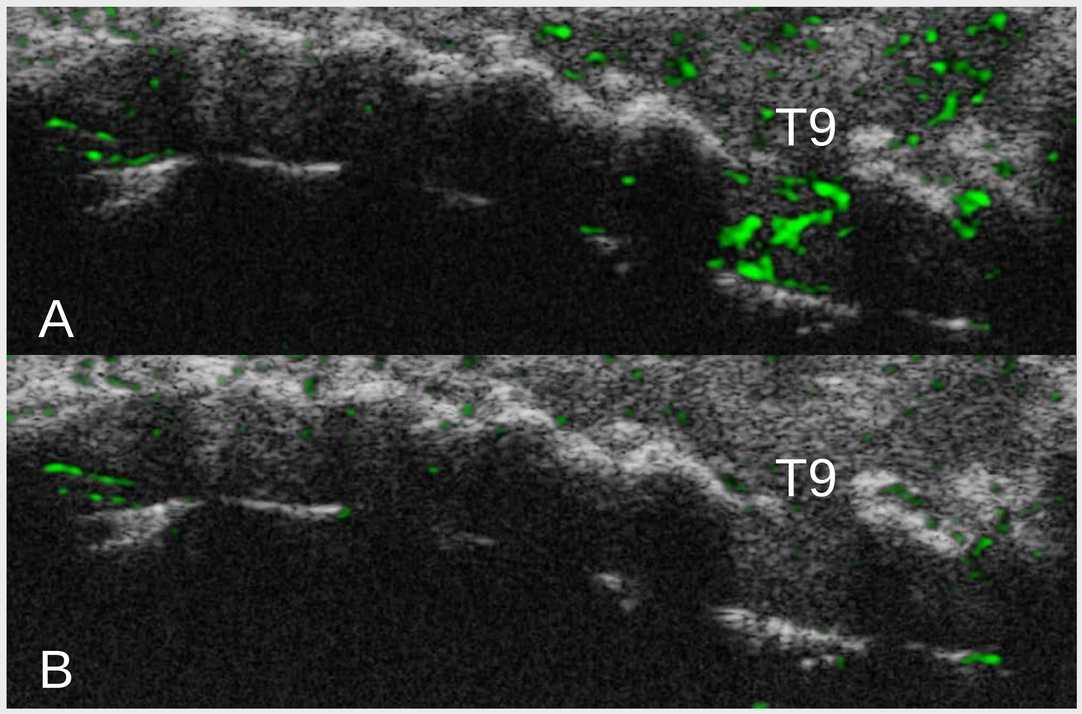
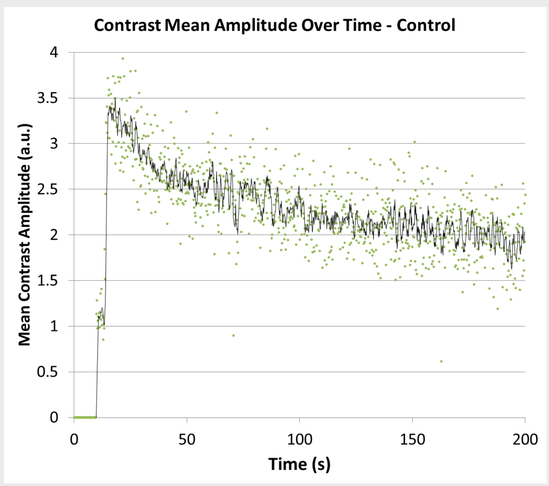


Figure 2. Sagittal images of the spinal cord, demonstrating accumulation of anti-P-selectin targeted contrast (A) before and (B) after the bursting pulse.

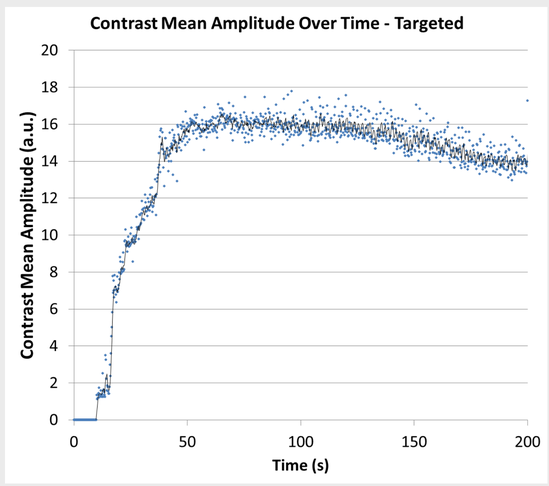
Isotype Control

Anti-P-Selectin

Wash in

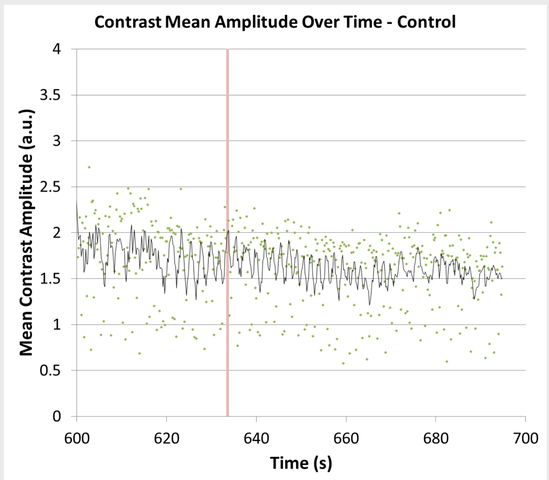


Graph 1. Control contrast wash in

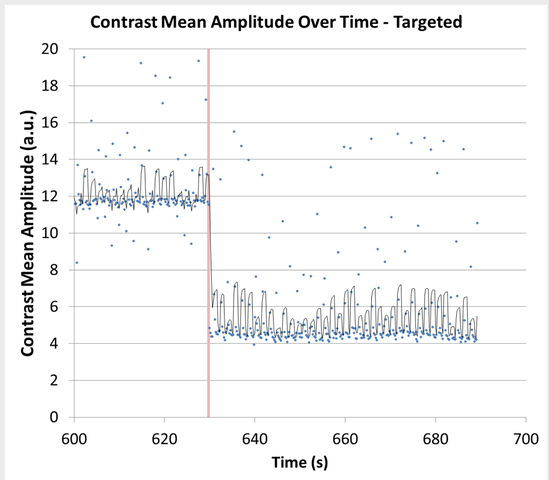


Graph 2. Targeted contrast wash in

Burst



Graph 3. Control burst results



Graph 4. Targeted burst results

Results

In the control contrast scan, the signal amplitude dropped quickly after peaking (Graph 1), while the contrast bound to anti-P-selectin showed a sustained peak and more gradual reduction in amplitude (Graph 2), This result is indicative of contrast binding. Trend graphs from mice injected with contrast bound to the isotype control antibody showed a stable signal amplitude after 10 minutes, with no significant reduction in amplitude after the bursting pulse (Graph 3). Trend graphs from mice injected with contrast bound to anti-P-selectin showed a significant decrease in signal amplitude following the bursting pulse (Graph 4). With any background tissue signal removed during image processing, the contrast signal amplitude is considered to be the sum of the signals from contrast bound to P-selectin and from circulating, unbound contrast[2]. The bursting pulse destroys all contrast microbubbles within the ultrasound beam profile, eliminating any bound bubbles, with a minimal reduction in circulating contrast[4]. Where binding occurs, a drop in the amplitude can be seen after the burst, due to the elimination of bound microbubbles (Graph 4). If no bubbles are binding, no drop in amplitude will occur, since the amount of bubbles in circulation is essentially unchanged (Graph 3). Results consistent with this explanation suggest that the anti-P-selectin targeted microbubbles were bound to expressed P-selectin in the inflamed neural tissue at T9.

Conclusions

This pilot study successfully demonstrated the ability of targeted CEUS to detect inflammatory markers in neural tissue. CEUS targeting of inflammatory markers has been accomplished in other soft tissues with microbubbles targeted to P-, L-, E-selectin, VCAM-1, and ICAM-1[5]. Following the protocol of this experiment, these efforts should be replicated in neural tissue, with a larger cohort, in order to determine which markers allow the most sensitive detection of neural inflammation. The inclusion of 3D contrast ultrasound imaging in future studies may offer a better understanding of global perfusion and inflammatory processes in the spinal cord beyond the site of injury.

References

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